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Short Report

Gap Junction Changes in the Supporting Cells of the Organ of Corti with IP_3

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Key words: organ of Corti, gap junction, Ca^{2+}, supporting cell, IP_3

Abstract

Gap junction conductance in the supporting cells of the organ of Corti is regulated by H^+ and Ca^{2+}. Studies with ryanodine and caffeine suggest that gap junction conductance in the supporting cells is regulated by calcium release from inside the cells. Since IP_3 plays a role in the physiological calcium release from the endoplasmic reticulum through IP_3 R. IP_3 was applied to the supporting cells of the organ of Corti to examine the physiological calcium releasing mechanism that affects gap junction conductance of supporting cells.

Introduction

Molecular biological and molecular genetic studies suggest that one cause of hereditary hearing loss may be related to the expression of connexin 26 [1–2]. Connexin 26 is distributed in the stria vascularis and supporting cells of the organ of Corti[3]. In a previous study, the author demonstrated the association of Ca^{2+} to gap junction conductance in supporting cells [4–9]. Here, this study investigates the changes in gap junction conductance in supporting cells resulting from the release of Ca^{2+} through the activity of IP_3.

The supporting cells of the organ of Corti are linked through gap junctions, according to electron microscopic, physiological and fluorescent studies [10–15]. Gap junction conductance of the supporting cells of the organ of Corti is controlled by H^+ and Ca^{2+} [4,5,16,17]. However, recent results have shown that Ca^{2+} is more effective than H^+ in regulating gap junction conductance of supporting cells as in other cells. The results of studies involving the application of ryanodine and caffeine to the supporting cells have led to the supposition that Ca^{2+} is released from intracellular calcium stores. Therefore, gap junctions of the supporting cells may be controlled by Ca^{2+} from both inside and outside the cells. My results also suggest the existence of ryanodine receptors in the calcium stores of supporting cells [6–8]. Ca^{2+} has been identified as a key component in cellular metabolism. IP_3 plays a role in stimulating the release of Ca^{2+} from endoplasmic reticulum. In the present study, physiological changes in the gap junction conductance of the supporting cells and the mechanism of IP_3–induced calcium release were investigated. By applying IP_3 to Hensen cells intracellularly, an attempt was made to uncouple the gap junctions of the supporting cells by intracellular calcium release from calcium stores.

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Methods

Albino guinea pigs were decapitated under halothane anesthesia and their cochleas were removed. The top two turns of each cochlea were separated and placed in a 750 μl perfusion chamber containing calcium-free Leibovitz salt solution with 500μg -1000μg/ml trypsin. The tissue was agitated gently in a shaker for about 15 min, and isolated cells were allowed to settle onto a coverslip placed at the bottom of the chamber. The cells were continuously perfused with ionic blocking solution containing 100mM NaCl, 20mM TEA, 20mM CsCl, 2mM $CoCl_2$, 1.52mM $MgCl_2$, 10mM HEPES, and 5mM dextrose, pH 7.4. Patch electrodes contained either 140mM CsCl or KCl, with 10mM HEPES, 1mM EGTA, and 2mM $MgCl_2$ at pH 7.4, with added IP_3 and AMP. AMP and $MgCl_2$ were left out of some of the solutions to compare the effect. The initial resistances of the patch electrodes were within 10 MΩ. An ionic blocking solution was used to reduce the voltage dependent ionic conductances, so that capacitive currents could be recorded in isolation. Blockers of plasmalemma voltage dependent ionic channels did not interfere with junction communication.

The measurement of input capacitance is a sensitive indicator of cell coupling because the input capacitance is proportional to the area of the electrically contiguous membrane. Changes in the input capacitance can therefore indicate the degree of cell coupling [17]. However , as other whole cell parameters may change during alterations in coupling, it is necessary to estimate the input capacitance that is corrected for these changes. The details of this estimation have been reported elsewhere [18].

Results

IP_3 was applied to the supporting cells intracellularly, as described above. The input capacitance remained stable during the initial 30 seconds after application of 10μM IP_3. Each supporting cells have capacitance which measured 24.9±9.19pF[15]. Usually, at 30 to 90 seconds after IP_3 application, the input capacitance decreased, due to an uncoupling of the gap junctions of the supporting cells. Figure 1 shows the effect of IP_3 application on the input capacitance in the presence of AMP and Mg^{2+}. Input capacitance decreased and series resistance increased, beginning at approximately 40 seconds after application. 12 cases out of 16 were measured capacitance changes. Approximately 75% (n=16) of the gap junctions were uncoupled in these experiments. As illustrated in Figure 2 , the application of 10μM IP_3, without added AMP but with Mg^{2+} resulted in an input capacitance and a series resistance that remained stable (n=6). Figure 3 shows that the gap junction conductance of Hensen cells remained stable in the presence of 10μM IP_3 without Mg^{2+} and with AMP. The ratio of gap junction uncoupling was only about 14% (n=7) in this experiment, but, with Mg^{2+}, the uncoupling ratio increased. Gap junction conductance decreased significantly with the application of 10μM IP_3 and AMP with Mg^{2+}. In the absence of AMP with Mg^2, the IP_3 effect decreased to about 33% (n=6). In the absence AMP without Mg^2 the IP_3 effect decreased to about 33% (n=3). All the numbers of all the groups are shown in Table 1. AMP has been reported to activate the release of Ca^{2+} from the calcium channel, and IP_3 was effective when applied with AMP[19]. Furthermore, the addition of Mg^{2+} with AMP, IP_3 was effective in uncoupling the gap junctions of the supporting cells.

Discussion

It has been demonstrated that the gap junctions in the supporting cells of the organ of Corti are controlled by H^+ and Ca^{2+}. Physiological levels of H^+ have also been found to control more effectively the gap junctions[20]. However, Ca^{2+} has proven to be more effective than H^+ in uncoupling gap junctions

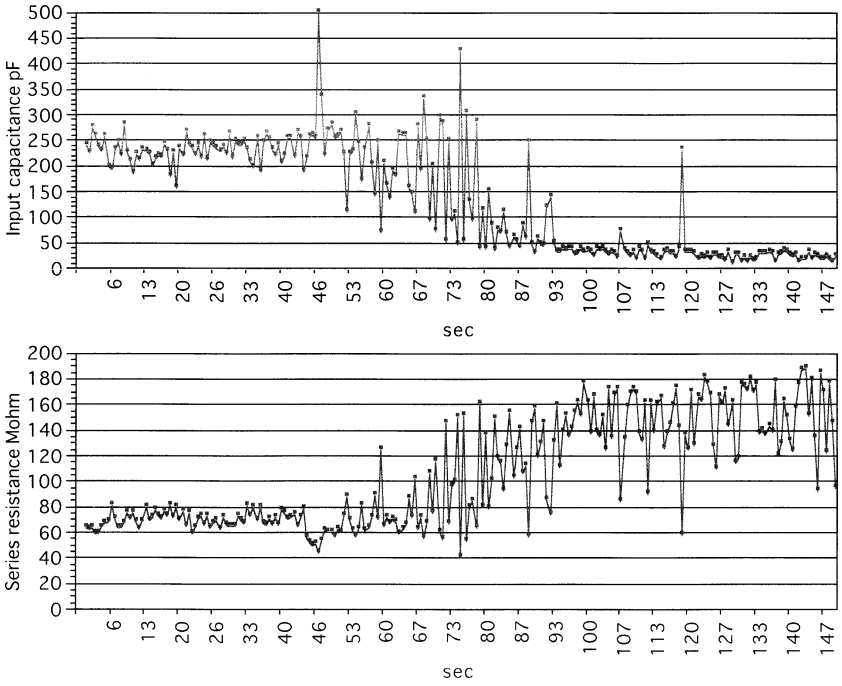

Fig. 1 10μM IP_3 was applied with AMP and Mg^{2+} to Hensen cells, intracellularly. Input capacitance decreased and series resistance increased. The gap junctions of the Hensen cells were uncoupled.

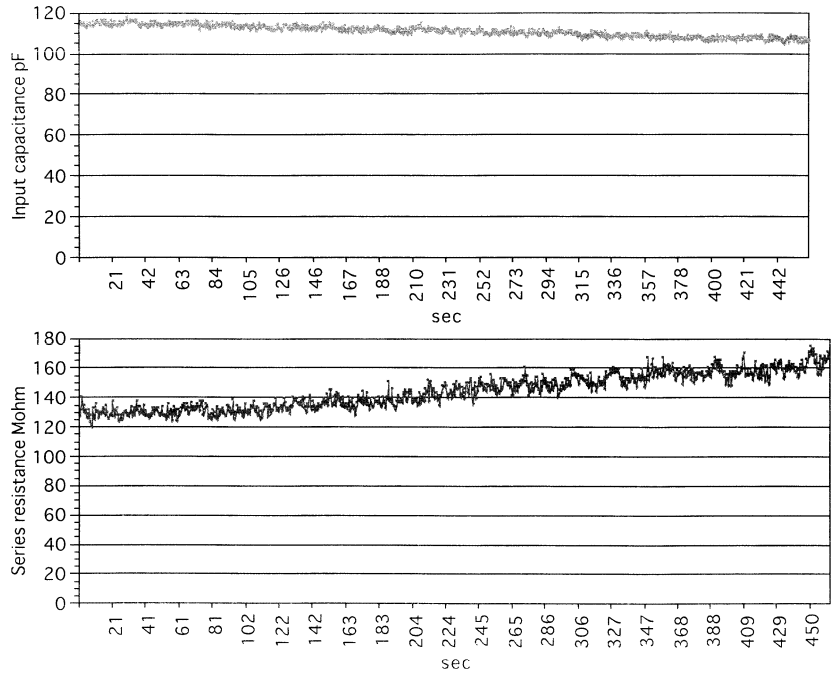

Fig. 2 10μM IP_3 was applied without AMP and with Mg^{2+} to Hensen cells, intracelluarly. Input capacitance and series resistance remained stable. The gap junction of the Hensen cells were coupled.

[21,22]. The results of experiments with ryanodine and caffeine suggest that the intracellular calcium releasing mechanism in supporting cells effectively uncouple the gap junctions. IP_3, which exists inside cells, is known to release Ca^{2+} from the endoplasmic reticulum. By applying IP_3 to supporting cells intracellularly, I suggested the Ca^{2+} releasing activity of IP_3. Furthermore, the data from IP_3 suggested

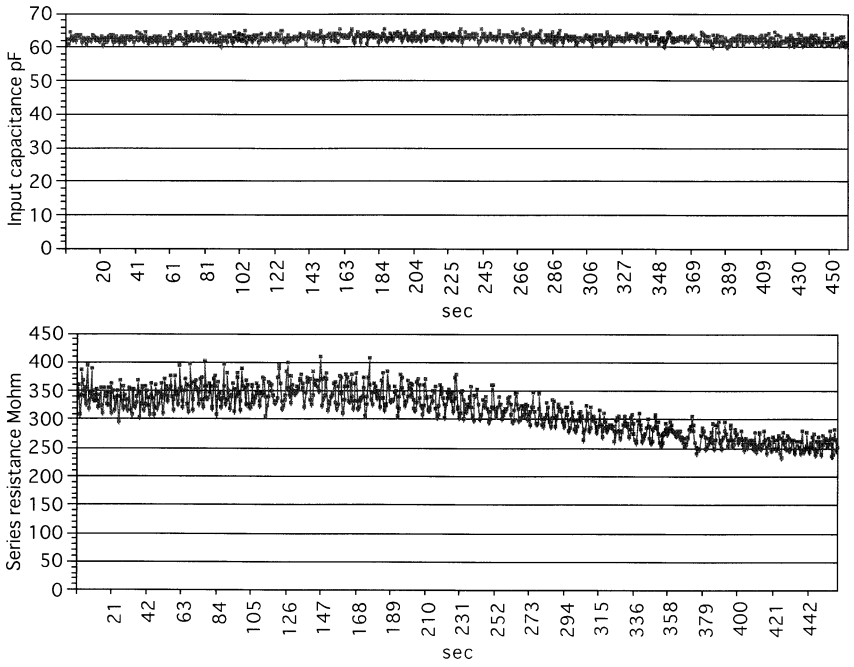

Fig. 3 10μM IP_3 was applied with AMP without Mg^{2+} to Hensen cells. The gap junctions of the Hensen cells were coupled.

Table 10μM IP_3 indicated that about 75% of the initially coupled Hensen cells were uncoupled with AMP and Mg^{2+}.

Effect of IP3

IP3, AMP (+) ,Mg (+)	IP3, AMP (−) ,Mg (+)	IP3, AMP (+) ,Mg (−)	IP3, AMP (−) ,Mg (−)
12／16	2／6	1／7	1／3

uncoupled/n

that AMP, together with Mg^{2+}, stimulates the release of Ca^{2+} from calcium channels inside cells. These results, regarding AMP and Mg^{2+}, indicate that adenylate cyclase activity occurs within cells.

In most cells, as well as the supporting cells, the endoplasmic reticulum is thought to function in calcium storage. In electron microscopic observations, a tubular structure was found to form a network in the supporting cells [23]. Based on my results, gap junction conductance may be regulated by Ca^{2+} originating from these intracellular calcium stores. Therefore, results from this study and others suggests that calcium release inside supporting cells plays a role in cell function and participates in the hearing mechanism of the organ of Corti [8,9].

Conclusion

Gap junctions of supporting cells of the organ of Corti are regulated by calcium release from inside the cells. IP_3 , with AMP and Mg^{2+}, stimulates the release of Ca^{2+} inside supporting cells.

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